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Introduction: Vitamin D (VTD) has been known for decades for its critical role in the phosphocalcic metabolism. Now, new clinical evidences have shown that VTD could play a major role in the prevention of different diseases (risk of falls, chronic and autoimmune diseases, cardiovascular diseases,...). Twenty-five hydroxy-vitamin D (25(OH)D) determination is now routinely prescribed in the Laboratory. Recently, different new methods have been available for this determination. Among them, LCMS/MS methods have emerged in some laboratories. However these methods are generally “home-brewed” and an important variability between them can be seen on different external quality controls, mainly due to a lack of standardization. Recently, Perkin-Elmer (Turku, Finland) launched a standardized method for 25(OH)D determination on LCMS/MS. This method is calibrated against the NIST 972 Standard and complies with the European In Vitro Diagnostic Directive. The aim of our study was to validate this method on the AbSciex TQ5500 (Framingham, Massachusetts, USA) (Figure1) LCMS/MS.

Materials and Methods: Briefly, in the Perkin Elmer method, 100 µl of serum sample, controls and calibrators [25(OH)D3-d6 and 25(OH)D2-d6] are precipitated in a solution of acetonitrile containing 0.1% formic acid and internal standard [25(OH)D3-d3 and 25(OH)D2-d3] and vortex-mixed. Then, 150µl of the supernatant are transferred into microplate wells and 50 µl are injected on a Brownlee SPP C18 chromatographic column. For the validation purpose, we determined the repeatability and reproducibility according to the CLSI EP-6A guideline, the functional sensitivity, the linearity. The accuracy profile was established on twelve serum pools containing different amounts of 25(OH)D3 (ranging from 11.5 to 111 ng/ml) in triplicate on five consecutive days. Finally, we evaluated the performance of the method on the last 13 DEQAS distribution, and the last 5 CAP proficiency testings.

Results: The intra assays CVS were: 5.2% at 11.5ng/ml, 2.9% at 25 ng/ml, 4.2% at 47 ng/ml and 2.1% at 111 ng/ml. The inter-assays CVs were 5.2% at 11.5 ng/ml, 3.7% at 25 ng/ml, 4.6% at 47 ng/ml and 3.5% at 111 ng/ml. The functional sensitivity was established at 1.54 ng/ml for 25(OH)D3. Between 11.5 and 111 ng/ml, the accuracy profile shows that the risk to fall out of the 13% acceptance limits is <5%, indicating that the method is validated in this range. The obtained linearity equation was: $Y = -0.0679 + 1.0007x$ with r^2 of 0.994 (Figure 2). Among the last 24 DEQAS controls that we were able to run, 19 and 5 results were comprised between the mean obtained by LCMS/MS users ± 1 and 2 standard deviation, respectively (Figure 3). And for the 5 CAP controls, we got the following results for D2: 10.2, <1.2, 7.7, <1.2, <1.2 and for D3: 8.1, 14.1, 20.3, 50.7, 40.2 while the mean results for the LCMS/MS users were: 10.6, <1.2, 7, <1.2, <1.2 and 9.7, 13.7, 24, 51.7, 37.5 for D2 and D3 respectively.

Figure 1

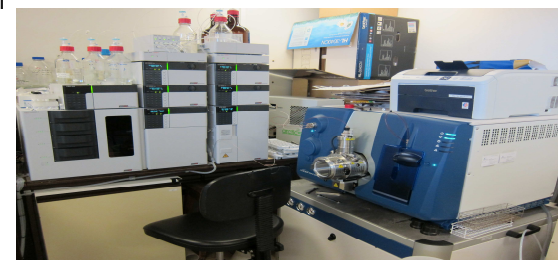


Figure 2

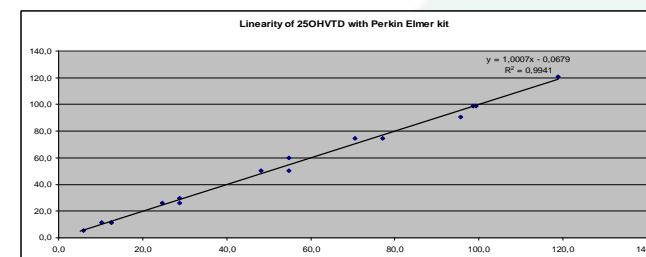
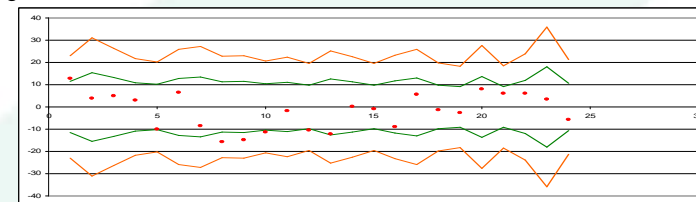


Figure 3



Conclusions: Perkin Elmer 25-OH vitamin D on the AbSciex TQ5500 is a robust method, with good CVs, linearity and a good recovery. It presents a good agreement when we compare the last DEQAS and CAP proficiency testings. The accuracy profile shows that the method is completely validated between 11.5 and 111 ng/ml.